REMARKS

Claims 1-6, 8-45 and 47-52 are pending in the application. Claims 27-33 and 48-50 are withdrawn from consideration in response to a Restriction Requirement. Claims 1-6, 8-26, 34-45, 47, and 51-52 are examined.

Drawings:

Applicants acknowledge that the Office has objected to the Drawings. Formal Drawings will be prepared and filed during continued examination as initiated by the accompanying Request for Continued Examination.

Specification:

Applicants acknowledge that the Office has reconsidered and withdrawn the previous objections to the specification relating to a typographical error and compliance with the Sequence Rules.

Claim Objections:

Applicants acknowledge that the Office has reconsidered and withdrawn the previous objections to claim 47.

Patentability Under 35 U.S.C. § 112, Second Paragraph:

Applicants acknowledge that the Office has reconsidered and withdrawn the previous rejections of claims 19-20, 22, 25, 26, 44, 45, and 51 under 35 U.S.C. § 112, Second Paragraph.

Patentability Under 35 USC § 102

Claims 1, 3-6, 8-18, 21-22, 24-25, 34, 37-39, 41-43, 51, and 52 are rejected under 35 U.S.C. 102(e) as allegedly anticipated by Belshe et al (US Patent 5,869,036). Belshe et al. is cited in support of the instant rejection, and likewise in support of the related rejection under 35 U.S.C. § 103, for allegedly teaching:

> an isolated cp-45 hybrid virus (a derivative of HPIV-3 JS) which is suitable for use as a vaccine in humans and animals comprising nucleic acid encoding nucleocapsid protein, phosphoprotein, at least one surface antigen of a target virus, and large polymerase protein, see

> columns 2-3. The target virus must have enveloped and have one or more surface antigens or surface glycoproteins (HN and F are surface glycoproteins), such as HPIV-1, HPIV-2 and RSV. Belshe et al disclose that the gene sequence which encodes the surface glycoproteins of the target virus may be substituted for the corresponding sequence in the cp45 genome which codes for the HN and F proteins, to result in a chimeric genome, see columns 8-9. Bovine RSV and cattle HPIV- are also included within the scope of Belshe et al. Attenuating mutations are introduced into the L segment as well as other proteins, see column 5, lines 42-67 and column 6, lines 1-3. Belshe et al disclose the use of their chimeric PIV in a vaccine, or immunogenic composition, comprising a physiologically acceptable carrier, see column 2, lines 32-33. Therefore, the claimed invention is anticipated by Belshe et al. (Office Action Paper No. 13, at pp. 5-6)

Applicants respectfully traverse the stated grounds for rejection and submit that the Belshe et al. reference neither teaches nor suggests the subject matter of the claimed invention.

Proper application of the Belshe et al. patent as an anticipatory reference under 35 U.S.C. § 102 places a direct burden on the Office to demonstrate that the reference discloses each and every element of the claimed invention. Continental Can Co. USA v. Monsanto Co., 20 USPQ2d (1991). In addition, any reference that is relied upon by the Office as anticipatory must fulfill all of the written description and enablement requirements of 35 U.S.C. § 112 commensurate with the rejected claims. As explained by the Federal Circuit in *In re Donohue*, 226 USPQ 619, (Fed. Cir. 1985).

> It is well settled that prior art under 35 U.S.C. § 102(b) must sufficiently describe the claimed invention to have placed the public in **possession** of it.
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> [E]ven if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling. (emphasis supplied, citing In re Borst, 45 USPQ 544, 557 (CCPA 1965), *cert. den.* 382 U.S. 973, 148, USPQ 771 (1966).

This requirement was applied in *Electronucleonics Laboratories, Inc. et al. v. Abbott Laboratories*, 214 USPQ 139, 147 (N.D. Ill. 1981) (underscore added, citations omitted).

> The standard for anticipation by patenting is the same one of a full enabling disclosure that applies to printed publications, i.e., it must disclose the invention in such full, clear and exact terms as to enable

> any person skilled in the art to which the invention relates to practice it.

The evidence of record, now including the accompanying Declaration of Dr. Brian R. Murphy, M.D. (incorporated herein by reference), clearly evinces that the Belshe et al. disclosure fails to meet the enablement standard for any recombinant PIV. By further extension, the reference clearly fails to enable recombinant, chimeric PIV useful as vectors and optionally incorporating one or more temperature sensitive mutation(s) or other attenuating modification(s) for use in immunogenic compositions and methods--as first produced and characterized in Applicants' disclosure. Likewise, the evidence presented herein and previously made of record establishes that the Belshe et al. reference fails to disclose or suggest the subject matter of the pending claims within the meaning of 35 U.S.C. § 103. These respective deficiencies are addressed collectively in the discussion below.

Importantly in the present case, the Office concedes that Belshe et al. fail to provide even a single working example of a recombinant PIV. The record is equally clear that the reference fails to provide any example of a recombinant, chimeric PIV having the particular modifications and useful properties disclosed in Applicants' specification. In this regard, it is understood that working examples are not *per se* necessary to meet the enablement requirements of 35 U.S.C. § 112, first paragraph. However, failure to provide working examples proving operability of an invention is an important factor to consider in determining enablement. In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988); Ex Parte Forman, 230 USPQ 546, 547 (BPAI 1986). This principle is embraced by the Patent Office, particularly in the context of unpredictable inventions, in MPEP § 2164.02, as follows:

> Lack of a working example, however, is a factor to be considered, especially in a case involving an unpredictable and undeveloped art.

Referring to the accompanying Declaration of Dr. Murphy, Applicants respectfully submit that their basic cDNA recovery method for producing recombinant PIVs, and particularly for generating recombinant PIVs for use in immunogenic compositions and methods, is neither taught nor suggested by the Belshe et al. reference (viewed in any combination with general knowledge in the art and/or the secondary

references of record). Dr. Murphy's Declaration sets forth detailed facts supportive of his conclusion rendered at ¶ 7 that:

> [T]he Belshe et al. patent (alternatively, the '036 patent) does not provide sufficient description and guidance to permit a person of ordinary skill in the art (at either the time the Belshe et al. patent or the current application was filed) to recover a recombinant parainfluenza virus (PIV) from cDNA. On the contrary, the Belshe et al. specification only hypothetically discusses the possibility of recovering a recombinant PIV from cDNA. No such cDNA nor recombinant PIV is actually described in structural terms that would qualify as a "written description" of such materials, and it is apparent that no "working example" of a cDNA encoding a recombinant PIV, nor of an actual recombinant PIV, is provided by the Belshe et al. disclosure. Additionally, considering the level of predictability in the art prior to the discoveries presented in the instant application, the teachings of Belshe et al. would not have been considered to provide an "enabling" disclosure of the presently claimed invention. That is to say, Belshe et al. offer such limited direction and guidance that, even when supplemented by available knowledge in the art at the time the instant application was filed, the skilled artisan would not have considered the public to be placed "in possession of" a recombinant PIV produced from cDNA. This conclusion, and the related conclusions below, relate to the fundamental technology of the present invention, i.e., successful recovery of a viable, self-replicating, recombinant PIV from cDNA. It is even more clear that certain detailed aspects of the invention, e.g., involving identification and manipulation of attenuating mutations and their introduction, singly and in combination, into a recombinant PIV, and successful recovery of chimeric PIVs, and attenuated chimeric PIVs, are neither disclosed nor suggested by Belshe et al.

Additional facts are presented in Dr. Murphy's Declaration to support the conclusion that Belshe et al. fail to render the presently claimed invention "obvious" within the meaning of 35 U.S.C. § 103. As stated by Dr. Murphy in paragraph 8 of his Declaration, "the limited teachings of Belshe et al. fail to provide a practical suggestion or motivation that would have led the skilled artisan to undertake production of a recombinant PIV from cDNA with a 'reasonable expectation of success'".

> Considering the poorly developed state of knowledge and high degree of unpredictability in the art, among other technical challenges discussed below, to achieve this goal without the benefit of the instant disclosure would have been viewed by the skilled artisan as requiring such extensive and uncertain experimentation that would have been characterized as "undue" experimentation--unattended by a

"reasonable expectation of success". The same facts, set forth below, that support this conclusion also point to a conclusion that the instant disclosure provides "unexpected results" in the successful production of a recombinant PIV from cDNA. Concerning more detailed aspects of the invention, the results provided by the instant disclosure allow production of singly and multiply attenuated, recombinant PIVs, chimeric PIVs, and attenuated chimeric PIVs, that are sufficiently infectious in a mammalian host to generate a desired immune response yet are suitably attenuated for selection and development as PIV vaccine candidates. That these novel results were achieved within the instant invention was even more surprising than the basic recovery of recombinant PIV, based on the limited teachings of Belshe et al. combined with general knowledge in the art at the time of the invention.

Applicants note that the Belshe et al. reference is limited in its teachings to a simple *in vitro* complementation assay described to evaluate temperature sensitivity. As Dr. Murphy points out in his Declaration, this limited study is attended by fundamental flaws in its design and interpretation. Nonetheless, even if the complementation assay results reported by Belshe et al. were accepted as valid, there is no reasonable scientific basis for extrapolating these findings into the context of a complete, infectious virus. In other words, these reported results cannot reasonably be extended to forecast a "reasonable expectation of success" for producing a recombinant PIV that is viable, attenuated, and immunogenic *in vivo* (Murphy Declaration at ¶¶ 10 and 12-13).

Briefly considering the deficiencies of Belshe et al. as noted by Dr. Murphy, it is readily apparent that the complementation assay described by Belshe et al. was a rudimentary system in which a plasmid expressing a wild type L protein in a transfected cell monolayer reportedly increased the level of replication of PIV3 cp45. The system notably failed to include a control plasmid containing the cp45 mutations, whereby the differences identified in the complementation assay cannot be ascribed to sequence differences between the wild type and cp45 PIV3 L protein, contrary to the assertions of Belshe and coworkers (Murphy Declaration at ¶ 11). In addition, the L cDNA employed by Belshe et al. was never evaluated for its effect on replication of wild type HPIV3. This is considered by Dr. Murphy to represent "a critical control" that would preclude the possibility that the observed increase in virus replication was simply due to increased expression of L protein (an aberration defined as a "dose effect" rather than true

complementation of a *ts* defect) (Id.) The assay of Belshe et al. (see Table 3) is also considered by Dr. Murphy to be unreliable in other crucial aspects. For example, reported positive results in the complementation assay contain internal inconsistencies and lack essential verification of important experimental controls. These inconsistencies would have been apparent to the skilled artisan and "would have detracted significantly from the motivation provided by the cited reference and the expectation of success that the artisan would have to practice the presently claimed invention as allegedly taught or suggested by Belshe et al." (Murphy Declaration at ¶ 12).

Yet additional evidence is provided in paragraph 13 of Dr. Murphy's Declaration that only as few as 3 or 4 cells, and certainly no more than 330 cells, in the complementation assay of Belshe et al. successfully produced virus, "whereas the remaining several million failed to produce a single particle." On this basis, Dr. Murphy concludes that:

> [E]ven if the complementation is accepted as authentic, it is of such a low efficiency that its significance is highly doubtful. In any case, there are no means for extrapolating findings from such a complementation assay to infectious virus. Thus, the 20-fold increase in the replication seen in the complementation assay cannot be extrapolated to predict or understand the magnitude of the contribution of the L protein mutations to the ts phenotype of cp45, nor what the biological properties of a hybrid virus carrying only these mutations might be. At best, the findings are simply suggestive that the L gene mutations might contribute to some undefined portion of the temperature sensitivity of the cp45 virus.

Also in paragraph 13 of his Declaration, Dr. Murphy notes that the complementation assay of Belshe "relates only to the *ts* phenotype of cp45, and does not address the attenuation phenotype *in vitro* or *in vivo*." While a *ts* phenotype often is associated with attenuation, it is not possible to predict that attenuation will indeed result, nor what its magnitude might be. This is considered by Dr. Murphy to represent "a critical deficiency" in the Belshe et al. disclosure, since the level of attenuation *in vivo* is an important requirement to developing effective immunogenic compositions and methods.

> Only by actually making an infectious recombinant virus, as provided in multiple working examples in the present disclosure, can one assess the *in vivo* attenuation of the virus to determine its usefulness as a

vaccine candidate. Again, each of these deficiencies of Belshe et al. would have been clear to the skilled artisan and would have undermined the motivation and expectation of success for practicing the claimed invention following the teachings of Belshe et al.

Dr. Murphy further states in paragraph 14 of his Declaration that:

> Only with the aid of the present disclosure providing a successful cDNA recovery system can the phenotypic effect of any desired mutation (e.g., an attenuating mutation from PIV3 cp45) be evaluated and demonstrated. For example the instant disclosure demonstrates that a ts mutation identified in L can be segregated from complementary or interactive effects of other cp45 mutations. In this context, it is critical for evaluating the speculative teachings of Belshe et al. that at least a representative set of mutations identified and segregated into a viable recombinant vaccine candidate be verified as attenuating, and that such attenuation be balanced sufficiently to yield a protective immune response in susceptible hosts. The simple studies of Belshe et al. were limited to complementation of replication for a cp45 virus using a wild type L plasmid. These studies were only conducted *in vitro* using tissue culture cells, and were not validated by parallel studies *in vivo*. In this context, it was quite possible that recombinant viruses incorporating one or more of the three "temperature sensitive" (ts) mutations in the cp45 L gene mutations would not be attenuating (att) *in vivo*. In particular, a finding that replication of cp45 may be complemented by wild type L protein in tissue culture cells is not clearly predictive that a virus bearing one or more of these mutations would be attenuated *in vivo*.

This correlative deficiency noted by Dr. Murphy is stated by him in paragraph 14 of his Declaration to be apparent from the following considerations. First, it is known that entire classes of viruses called "temperature-dependent hose range (td-hr) mutants" may be ts on one tissue but not on other tissue culture cells. These td-hr mutants are not necessarily attenuated *in vivo* (see Snyder et al., Virus Research 15:69-84, 1990; and Shimizu et al., Virology 124:35-44, 1983). As described in Snyder et al., an exemplary mutant (clone 143-1) of influenza virus was shown to be highly ts in tissue culture cells, but was not significantly attenuated *in vivo*. Additional findings by Shimizu et al. indicate that such td-hr mutants are common and are found in many different complementation groups of the influenza virus (i.e., they are present in many different genes of the virus). Based on this and other evidence, Dr. Murphy renders the following conclusions:

> The Belshe et al. reference does not demonstrate whether any of the contemplated ts mutations in the L gene of cp45 belong in the td-hr class of mutations or in the other class of ts mutations whose replication is effected by the temperature present in the host animal. In view of this deficiency, the simple description of a complementation phenotype for a group of multiple, unsegregated mutations in a complete gene *in vitro* does not serve as a reliable indicator of attenuation *in vivo*. As detailed herein, the instant disclosure provides the basic tools, along with fully detailed description and guidance, to resolve these deficiencies and enable the skilled artisan to practice the invention throughout the scope of the claims presented for review.

Additional discussion is provided in paragraphs 15 and 16 of Dr. Murphy's Declaration pertaining to the requirement for fidelity in determining and describing sequence information for cDNAs and plasmids to allow initial generation of a recombinant virus and validation of a successful recovery system. It is noted that the sequence of the cp45 L gene provided by Belshe et al. was taken directly from published work by others and were previously considered possible attenuating mutations. Those sequences were subsequently found to contain errors, whereas the present application provides the complete, correct sequence of cp45. This information complements the first successful attempt to generate recombinant, chimeric PIVs that are successfully employed as vectors, which optionally incorporate one or more attenuating mutations in the genes encoding the PIV L, C, and F proteins. The present work also provides the first phenotypic analyses and related characterization for these novel recombinant PIV mutants.

Examination of Figure 1 of Belshe et al. fails to reveal a useful mutation in the PIV C protein. The Figure instead reiterates incomplete sequence information and analysis previously reported by Stokes et al. From Belshe et al., one would not know that the F or C mutations present in cp45 were attenuating mutations, and that these mutations are useful in cDNA-derived recombinant, chimeric PIV vector constructs. In contrast, the materials and methods provided in the instant disclosure correctly identifies a full complement of attenuating mutations present in cp45, as well as representative attenuating mutations identified in heterologous viruses (e.g., a mutation designated 456 from the respiratory syncytial virus (RSV) L protein, a mutation designated 170 from the Sendai virus C protein, and a mutation designated 1711 mutation from the L protein of

bovine PIV3 (BPIV3)) for incorporation into recombinant chimeric vector PIVs of the invention. As summarized by Dr. Murphy at paragraph 15 of the Declaration:

> In this context, the Belshe et al. disclosure provided little new information on the nature of the genetic determinants of the ts phenotype of cp45--only following previous suggestions that one or more mutations in L might specify some portion of the ts phenotype in cp45. In contrast, by describing successful recovery of recombinant PIV from cDNA, and by further incorporating individual and combinatorial mutations from cp45 (from several genes as well as from extragenic portions of the genome) in recombinant PIVs, the instant disclosure dissects and maps out the specific contributions of the individual lesions in cp45 to the attenuation phenotype. Following introduction of these various, representative mutations, singly and in combination, into recombinant PIVs, the ability to achieve an attenuation phenotype using various manipulations, and to fine tune the attenuation phenotype to achieve useful vaccine strains, was established using widely accepted *in vivo* models for attenuation and immunogenic activity in humans.

As noted above, recovery of a recombinant virus from cDNA was not accomplished by Belshe et al. Nonetheless, the reference speculates even further concerning the prospect of chimeric "hybrid" recombinant vaccine viruses (see, e.g., Example 7). However, Belshe et al. clearly fail to describe or provide specific guidelines for construction of a chimeric vector PIV cDNA construct. Nor does the reference describe and enable methods for recovering such chimeric vector constructs, or provide actual data characterizing a chimeric vector PIV *in vitro* or *in vivo* for identifying candidates useful in immunogenic compositions and methods. As summarized by Dr. Murphy in paragraph 17 of his Declaration:

> Thus, although the principal disclosure of Belshe et al. purports to render construction of chimeric PIV and other "hybrid" viruses possible, the reference neither describes, teaches nor suggests the presently claimed subject matter. On the contrary, no specific guidance is provided to enable any kind of cDNA recovery of PIV, much less recovery of a viable, attenuated and infectious chimeric PIV as provided by the instant disclosure. The speculative teachings of Belshe et al. would not have been accepted by the skilled artisan as providing a clear teaching or practical motivation to achieve the presently claimed invention. This conclusion is underscored by the vast diversity of viral "targets" contemplated by Belshe et al. for constructing "hybrid" viruses . . ..

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> In contrast to these broad, prophetic and overreaching statements, the present specification provides detailed description and guidance, as well as a fully representative assemblage of working examples (e.g., various attenuated PIV3-1 chimeric vaccine candidates) that is fully commensurate with the scope of claims presented for review.

Additional differences between the present disclosure and the Belshe et al. reference relating to the description of a system to recover infectious replicating viruses from cDNA for selection as vaccine candidates are outlined in Tables 1–3 of Dr. Murphy's Declaration, and are briefly addressed in the subsequent paragraphs. Referring to Table 1 and paragraph 18 of the Declaration, it is noted that Belshe et al. fail to provide an accurate sequence of a wild type PIV virus. This is considered by Dr. Murphy to represent "a critical deficiency for describing and enabling the instantly claimed invention." Relating to this conclusion, PIV lacks a proof-reading polymerase and is known to have a high error rate. During cDNA cloning this high error rate is reflected in a relatively large number of sequence differences among clones, which are heightened by additional errors introduced during RT, PCR, and propagation in bacteria. A single point mutation in the 15.4 kb sequence can be sufficient to preclude recovery, and the identification and correction of potential errors presents "a formidable challenge." The sequences described in the prior art and incorporated by Belshe et al. were later modified to correct errors, and the ultimate recovery of infectious virus verified that the presently described sequence is "viable". Commenting on these facts, Dr. Murphy concludes as follows:

> Thus, Belshe et al. rely on the previously reported sequence by Stokes et al, and there was no evidence at the time that this sequence, shown in the present disclosure to be inaccurate, could have yielded a viable virus. Even if this untested, incorrect sequence were employed successfully to obtain a recombinant virus, it was nonetheless unpredictable whether the sequence would specify a replication competent phenotype, i.e., a level of replication compatible with immunogenicity *in vivo*. Thus, Belshe et al. would not have been considered by the skilled artisan to enable recovery of a recombinant PIV3 nor a chimeric vaccine virus, since there was insufficient evidence that the reported sequence would yield these required results. Only the instant disclosure provides an authentic sequence of the full length PIV3 and its contiguous sequences in a plasmid with correct T7

> promoter elements, T7 terminators, and hepatitis delta ribozyme. It is noteworthy that during the nearly seven years after the filing date of Belshe et al., Belshe and coworkers have apparently failed to recover any PIV from cDNA. In contrast, the instant disclosure provides a large, fully representative panel of recombinant viruses, including singly and multiply attenuated viruses and chimeric viruses. Among these recombinant viruses, PIV3 and PIV1 viruses and chimeric "vectors" have been constructed and demonstrated to be suitably attenuated and immunogenic to yield protection against PIV1, PIV2, and PIV3. Following these detailed teachings, our lab has progressed into clinical studies for PIV vaccine candidates recovered from cDNAs.

As further clarified by Table 1, and paragraph 20, of the Murphy Declaration, Belshe et al. also fail to describe or enable any specific sequence for an "insert" to yield a chimeric virus that would be compatible for efficient replication in a PIV3 backbone. Instead, Belshe et al. simply reference viral proteins, but do not specify any specific sequence of an insert, nor an insert length (see Belshe et al., columns 17– 18). Dr. Murphy concludes that "[s]ince there were many sequence errors existing in the literature, it would not have been possible to determine whether the chimeric viruses prophetically reported by Belshe et al. would be viable, or, if viable, would possess a replication competent phenotype, i.e., a level of replication compatible with immunogenicity *in vivo*." In contrast, the PIV1 sequences used in the construction of chimeric cDNAs of the present invention to generate a PIV3-1 vector virus were obtained from a wild type PIV1 of known virulence for humans and, following insertion into the PIV3 backbone, yielded a chimeric virus with a verified wild type phenotype. Dr. Murphy summarizes the attendant deficiencies of Belshe et al. as follows:

> The genes that encode the proteins alluded to by Belshe et al. include gene start sequences, a 5' non-coding region, coding region, 3' non-coding region, and gene-end sequence. The exact junctions of the sequences for the inserts referred to by Belshe et al. were not described and therefore one would not have known from the Belshe et al. description whether to include any of the extra-coding sequences or not. For example, the genes from any given virus contain transcription signals that differ from those of another virus, yet it is essential that the "transferred" gene be faithfully expressed in the new, heterologous viral backbone. This critical issue is not even addressed in the Belshe et al. specification. In contrast, the instant disclosure provides exemplary descriptions of an insert, backbone, transcription signals

and junctions to yield viable chimeric PIVs that are useful vaccine candidates.

Yet another critical deficiency of Belshe et al. that is considered by Dr. Murphy to be resolved by the present disclosure relates to the length of the viral genome for production of recombinant PIVs (Murphy Declaration at ¶ 21). The length of the PIV genome needs to be an even multiple of six in order to recover authentic copies of virus containing the exact sequence in the cDNA. This "rule of six" reflects the association of each NP monomer with six nucleotides. If the genome does not conform to the rule of six, mutant viruses are recovered that have random mutations that correct the length. According to Dr. Murphy:

> [T]his factor adds a major aspect of uncertainty to the teachings of the Belshe et al. reference, which fails to appreciate the significance of the rule of six and the errors that would arise by failure to properly construct cDNAs in accordance with this requirement." In the instant disclosure, the exact lengths of a full length cDNA for PIV3 (number of nucleotides = 15462) and for PIV3-1 (number of nucleotides = 15516) are provided. This description in turn depended on the actual, successful recovery of recombinant PIVs and subsequent analysis and verification of the fidelity of the viral sequence and phenotype.

As further emphasized by Dr. Murphy, the specific methods used to recover infectious virus also need to be described to enable production of recombinant viruses from cDNAs. Systems to recover negative stranded RNA viruses such as PIV from cDNA are complicated and require a suitable cell capable of both successful transfection by plasmids and replication of the rescued virus. Dr. Murphy notes that "the recovery of infectious recombinant negative stranded RNA viruses is generally quite inefficient", such that out of 1,000,000 transfected cells, 10 or fewer cells actually produce virus. Particularly for a human pathogen such as PIV3, which does not grow rapidly *in vitro*, it is "a formidable challenge" to successfully produce and recover recombinant virus from cDNA. Considering these factors, Dr. Murphy states that:

> Our studies confirmed that the precise amount of the viral cDNA and support plasmid DNA was critical for initial recovery of recombinant PIV, and this factor was not appreciated by Belshe et al., who failed to even initiate a recovery system. As another example of inadequate guidance, Belshe et al. describe prophetically the use of cDNA expressing a genome sense RNA to recover virus (column 10, line 35). It is now known, however, that for technical reasons the recovery of

> virus from genome-sense RNA is relatively inefficient at best, and often unsuccessful. An optimal strategy employs a cDNA expressing a positive sense copy of the genome (called an antigenome). This guidance is clearly provided in present disclosure.

In paragraph 23 of his Declaration, Dr. Murphy focuses on the requirement for a complete description of a system to promote expression of viral proteins from support plasmids and from a full-length cDNA to form a functional transcriptase/replicase/genome complex, without which disclosure production of a recombinant PIV from cDNA would not be enabled. Such a "full description" of this system is considered by Dr. Murphy to be lacking in the disclosure of Belshe et al. For example, the system described by Belshe et al. uses a replication competent vaccinia virus expressing T7 (Column 15 of Belshe), but it does not specify how a viable PIV virus would be recoverable in the presence of a vast excess of fully infectious, replication-competent vaccinia virus. Dr. Murphy concludes that "[I]t is unlikely that a low concentration of a recombinant PIV could be biologically separated from the replication-competent vaccinia." This is especially true since vaccinia virus is highly permissive for most cell types and is extremely difficult to fully neutralize with antibody. In contrast, the methods described in the instant disclosure recognize and employ a replication deficient vaccinia virus (MVA-T7). This adaptation permitted the successful recovery of a recombinant PIV in the presence of the MVA-T7.

> Thus, Belshe et al. did not describe a system that would have been considered capable of successfully recovering recombinant PIV from cDNA, particularly attenuated (or attenuated, chimeric) viruses having further restrictions on replication. (Murphy Declaration at ¶ 23).

Additional discussion provided in Dr. Murphy's Declaration (at ¶ 24) points to other significant "complexities and uncertainties" for successful recovery of PIV from cDNA that were not overcome by Belshe et al. In particular, Dr. Murphy cites unsuccessful work by others in the field attempting to achieve recovery of other infectious recombinant negative strand RNA viruses. For example, despite extensive foundational research and discovery aimed at recovering a recombinant measles virus, successful production of the recombinant measles virus from cDNA was not reported until 5- to 6-year after Ballart et al. (EMBO J. 9:379, 1990) reported construction of a

complete cDNA expressing the genome of measles virus under the control of a T7 promoter and the recovery of recombinant virus by complementation of this synthetic genome with intracellularly-expressed measles virus proteins. Notably, this report, which parallels in certain aspects the prophetic disclosure of Belshe et al., proved to be in error and was retracted. The long delay in achieving a successful measles virus recovery system after the general strategy for recovery was mapped out by Ballart and coworkers underscores "the formidable technical and conceptual challenges that must be met to achieve a successful recovery system" for any negative stranded RNA virus (Murphy Declaration at ¶ 24). In fact, at this stage of development in the art, "there was genuine concern that successful recovery of any negative strand virus might not be feasible." Thus, the successful recovery of recombinant rabies rhabdovirus in 1994 (Schnell et al 1994 EMBO J. 13:4195) was a "major milestone" (Id.) However, it was not immediately clear whether this would be successful with paramyxoviruses, which have substantially greater genome size and complexity, more complex sets of protein products, and poorer growth and stability. In work with a second virus, the highly efficient rhabdovirus vesicular stomatitis virus (VSV), it was shown in 1990 that plasmid-expressed proteins could support a biologically derived nucleocapsid (Pattnaik et al, 1990 J. Virol. 64:2948), but two more years were required to develop the capability to express a defective interfering particle from cDNA (Pattnaik et al, 1992 Cell 69:1011) (Id.) Three more years were required to express complete infectious recombinant virus (Lawson et al 1995 Proc. Natl. Acad. Sci. USA 92:4477; Whelan et al, 1995 Proc. Natl. Acad. Sci. USA 92:8388), which also was viewed as "a major achievement" (Roberts and Rose 1998 Virology 247:1). The work with the rhabdoviruses rabies and VSV involved unexpected requirements, such as the need to express the genome in positive sense form, the need to avoid structures causing early termination by the T7 RNA polymerase, and the need to reduce the background of vaccinia virus. Summarizing these developments, Dr. Murphy states as follows:

> In many instances, recovery depended on methods that could not be applied generally to other viruses, such as removal of the vaccinia virus background by filtration (Schnell et al 1994 EMBO J 13:4195, Lawson et al, ibid), which could not be applied to paramyxoviruses because of their large size and hence necessitated the development of alternative strategies. Studies in other nonsegmented negative

> stranded viruses illustrate still other unexpected requirements, such as the need to express an additional protein, the M2-1 protein, to achieve successful recovery of human respiratory syncytial virus (Collins et al, 1999, Virology 259:251). This brief survey of the literature embraces only to a subset of studies I know to have been undertaken in large numbers of labs across the globe seeking to recover negative stranded RNA viruses from cDNA. Many of those labs that never came close to successful recovery, thus their efforts have gone unreported. In summary, myriad challenges have persisted in the art to development of a successful recovery system for PIV. These challenges underscore the deficiencies of Belshe et al., who provide only vague, generic concepts without documentary experimentation nor demonstration of a feasible recovery system for PIV. At the same time, the slow-developing state of the art, and the high level of unpredictability in the field, emphasize the unexpected nature of results provided within the instant disclosure.

In the closing paragraphs (¶¶ 25-26) of his Declaration, Dr. Murphy briefly contrasts the instant disclosure with that of Belshe et al. in the context of characterizing the *in vivo* activity of a recombinant PIV recovered from cDNA. In this context, Dr. Murphy emphasizes that "[t]he properties of the virus that make it a successful vaccine candidate must be described in detail in a representative assemblage of recombinant species, as provided by the instant disclosure." He points to three possible consequences that can occur when one attempts to recover a wild type PIV3 or a chimeric recombinant virus from cDNA: (1) a recombinant virus is recovered that replicates to levels characteristic of wild type virus or indicative of attenuation; (2) a recombinant virus is recovered that contains one or more inadvertent and unknown sequence errors that render it defective in any of a number of ways; and (3) virus is not recovered, due either to one or more lethal sequence errors or some deficiency in the recovery strategy or conditions. In addition, when one introduces mutations into such a cDNA intended to attenuate the virus and thereby to render it useful as a vaccine candidate, at least four outcomes are possible: (1) one can increase the virulence of the virus; (2) one can incompletely attenuate the virus; (3) one can achieve a satisfactory level of attenuation such that a virus can be used as a vaccine; and (4) one can over-attenuate a virus or render it non-viable. In reference to the current specification, Dr. Murphy states that:

> The examples provided in the instant specification fulfill criterion 3 by providing a representative assemblage of recombinant viruses that are suitably attenuated for development as vaccine agents. In contrast, the

> disclosure of Belshe et al. fails to achieve any of the foregoing possibilities--by virtue of its failure to describe and enable a cDNA construct encoding a recombinant PIV (see Table 1), for failing to recover infectious virus from cDNA (Table 2), and for the lack of testing and characterization of an infectious, recombinant virus (Table 3).

By way of further contrast, the Belshe et al. specification provides only a limited description concerning the use of a plasmid expressing a wild type PIV3 L protein to enhance replication of a JScp45 virus at a restrictive temperature of 39.5°C. According to Dr. Murphy:

> This limited disclosure does not provide a reasonable scientific basis for the speculation by Belshe et al. that the L gene of cp45 possesses mutations that might be useful in a recombinant PIV vaccine virus derived from cDNA. The virus recovered by Belshe et al. after complementation with the L-encoding plasmid at the restrictive temperature was <u>not</u> changed or modified in any manner as contemplated by Applicants' disclosure. No cDNA constructs were designed and produced from which PIV3 wild type viruses could be recovered, and certainly no new constructs or recombinant viruses bearing a chimeric genome or antigenome, and/or specific, attenuating mutations were described or enabled. The absence of such disclosure in the Belshe et al. reference negates any "reasonable expectation for success" to achieve the presently claimed invention in either its independent or dependent aspects. This is especially clear when the particular results provided by the instant disclosure are appreciated, namely that it was shown to be possible to construct a panel of recombinant PIVs, including singly and multiply attenuated and chimeric viruses, from cDNA that are suitably attenuated and immunogenic for development as vaccine candidates.

In view of the foregoing evidence and remarks, Applicants respectfully submit that the disclosure of Belshe et al. does not teach or suggest production in a cDNA-based recovery system of any infectious PIV, and particularly a recombinant, chimeric vector PIV that is optionally attenuated by incorporation of one or more recombinantly introduced mutation(s) as disclosed in Applicants' specification. Nor does the Belshe et al. reference teach or suggest yet more challenging aspects of Applicants' invention, such as production of infectious, multiply attenuated and attenuated, chimeric vector PIVs for use in immunogenic compositions and methods. The simple complementation assays described by Belshe et al. using the biologically derived mutant cp45 virus and a wild

type L plasmid, was only conducted *in vitro* using tissue culture cells, and was not validated by parallel studies *in vivo*. From this disclosure, the report that replication of cp45 can be complemented by wild type L protein in tissue culture cells does not amount to a reasonable scientific forecast that a recombinant virus bearing one or more of the cp45 mutations would be attenuated *in vivo*.

This correlative failure and other deficiencies of the Belshe et al. reference noted in the record clearly evince that the alleged teachings of Belshe et al. are inoperable for production of a recombinant PIV from cDNA, and that the reference fails to provide a "practical motivation" and "reasonable expectation for success" to render the presently claimed invention obvious within the meaning of 35 U.S.C. ¶ 103. This determination correlates with evidence presented in the record that the skilled artisan would not have had a reasonable expectation of success to follow the teachings of Belshe et al., with the application of available knowledge in the art, to achieve the instantly claimed invention.

Finally, even if one considers that the Belshe et al. reference may have provided a suggestion to attempt recovery of recombinant PIV from cDNA, the facts of record establish that such suggestion amounted, at best, to an "invitation to experiment", whereas the results set forth in Applicants' disclosure clearly represent "unexpected results" sufficient to overcome any prima facie case of obviousness based on the art of record. In view of these and other facts and authority presented in the record, Applicants respectfully submit that the rejection of claims 1, 3-6, 8-18, 21-22, 24-25, 34, 37-39, 41-43, 51, and 52 under 35 U.S.C. 102(e) as allegedly anticipated by Belshe et al (US Patent 5,869,036), has been overcome.

Patentability Under 35 USC § 103

Claims 1-6, 8-26, 34-45, 47 and 51-52 are rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Belshe et al in view of Collins et al (US Patent 6,264,957) and Klein et al (W093/14207).

The teachings of Belshe et al. are relied upon by the Office as described above. Belshe et al. is further cited for allegedly teaching "attenuating mutations in the HPIV3 L protein wherein leucine is substituted for phenylalanine". (Office Action Paper No. 13, at p. 7). The Office notes that Belshe et al. does not teach where the heterologous gene

segments are added, a subviral particle, an attenuating mutation at position 456 of HPIV3 L protein, or a mutation stabilized by multiple nucleotide changes in a codon specifying the mutation. It is also noted by the Office that Belshe et al. do not teach an immunogenic composition dosage and route of administration.

Collins et al. is cited for allegedly teaching "RSV vaccines comprising subviral particles."

Klein et al. is cited for allegedly teaching "a multimeric hybrid gene, comprising RSV (G or F protein) and HPIV (F or HN protein), and combinations of these proteins such as F proteins from both PIV3 and RSV". In addition Klein et al. is cited for teaching "a vaccine formulated for administration intranasally."

On the basis of these combined disclosures, the Office asserts that one of ordinary skill in the art "would have been motivated to modify the chimeric PIV of Belshe et al. by substituting subviral particles because it was known in the art at the time of the invention that subviral particles are effective in vaccine compositions as taught by Collins et al." (*Id.*, at pp. 7-8). Furthermore, the Office contends that "[o]ne of ordinary skill would know the dosage required to elicit an immune response and would have been motivated to make the modifications of dosage and administration in order to achieve the maximum immune response." It is also asserted by the Office that a person skilled in the art "would also know where to add the heterologous gene segment given the well-known art of recombination and would have been motivated to incorporate the segment in such a way as to ensure its expression and stability" Finally, the Office asserts that "Belshe et al. teach the method of incorporating the heterologous (target gene clone) segment by ligation into the PIV clone."

Applicants respectfully traverse the foregoing grounds of rejection and submit that the invention of claims 1-6, 8-26, 34-45, 47 and 51-52 is neither disclosed nor suggested by the combined teachings of Belshe et al., Collins et al. and Klein et al. viewed for what they teach as a whole.

The fundamental grounds stated in support of the instant rejection are based on the limited teachings of Belshe et al., as discussed above. In this regard, the Office contends that it would have been obvious to "modify the chimeric PIV of Belshe et al." using the secondary teachings of Collins et al. and Klein et al. However, as noted above,

Belshe et al. do not disclose a "PIV clone" that would be a potential subject for modification in accordance with the Office's proposal. On the contrary, as discussed in detail in the preceding section, Belshe et al. neither describes nor enables such a recombinant PIV clone. It is even clearer from the preceding discussion that this primary reference does not teach nor suggest methods and compositions that would be useful for generating live, attenuated chimeric vector PIVs for use in immunogenic compositions and methods commensurate with Applicants' claimed invention. Instead, the Belshe et al. reference provides, at best, an invitation to experiment toward the production of "hybrid" viruses selected from "any enveloped virus that has one or more surface antigen."

For these reasons, the Belshe et al. reference is notably deficient as a primary reference that would satisfy the Office's interpretation. The secondary teachings of Collins et al. (relating to "RSV vaccines comprising subviral particles", and of Klein et al., relating to "a multimeric hybrid gene" between RSV and HPIV glycoproteins, and "a vaccine formulated for administration intranasally"), clearly fail to rectify the noted deficiencies of the primary reference. In particular, these secondary teachings do not supplement the teachings of Belshe et al. in a direction or manner that would provide the requisite "reasonable expectation of success" for producing live, attenuated, chimeric vector PIV viruses, suitably attenuated for use in immunogenic compositions and methods. Accordingly, the rejection of claims 1-6, 8-26, 34-45, 47 and 51-52 under 35 U.S.C. 103(a) over Belshe et al. in view of Collins et al. and Klein et al. is believed to be overcome.

CONCLUSION

In view of the foregoing, Applicants believe that all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes that a telephone conference would expedite prosecution of this application, please telephone the undersigned at (425) 455-5575.

Attached hereto is an appendix detailing the status of claims.

Date: April 30, 2003

Respectfully submitted,

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